SUPPORTING INFORMATION

In vitro Antiviral Activity of New Oxazoline Derivatives as Potent Poliovirus Inhibitors

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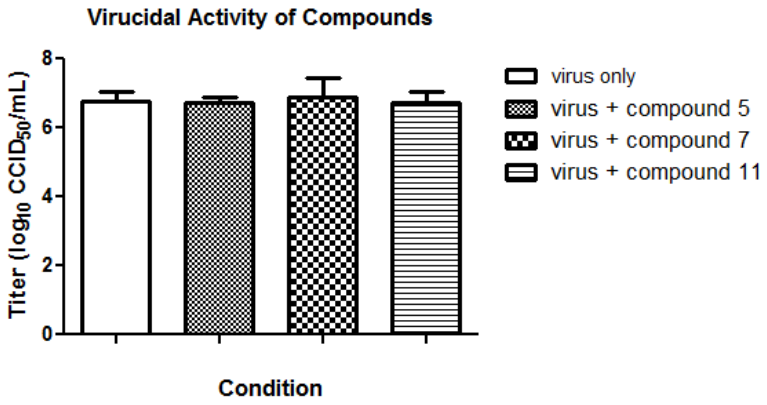
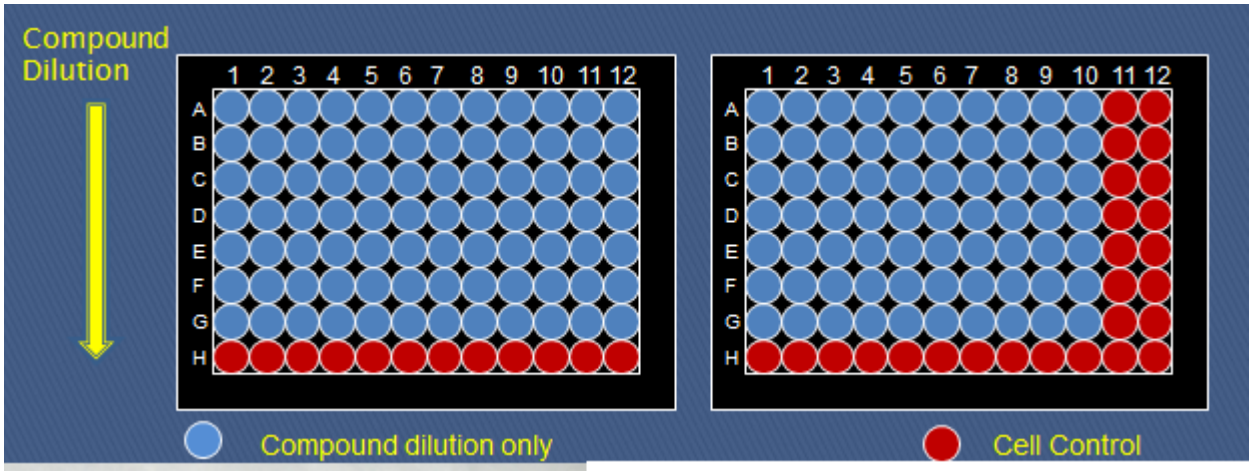
Contents:

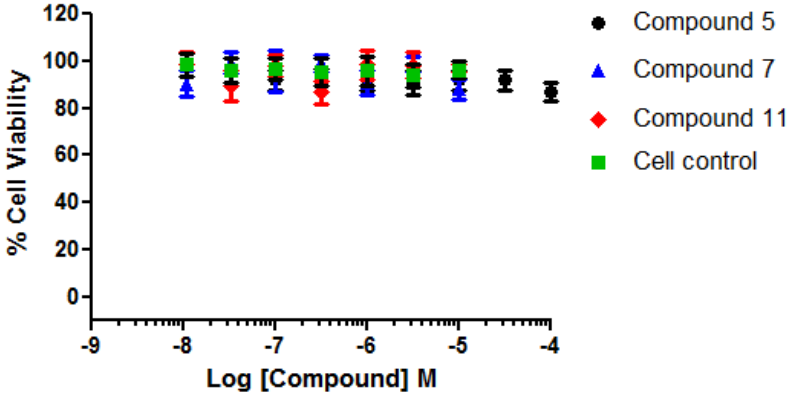
1. Virucidal activity of compounds 5, 7 and 11.



Virucidal activity of compounds. Compounds at 1 μM were incubated with virus (Sabin 1) for 2 hours at 37˚C. The virus–compound mixture was diluted 100-fold and added to HeLa cells at conditions previously described. At this dilution, the compounds were present at less than effective concentration (0.01 μM) while allowing virus CPE to proceed. Using this experimental format, a virucidal effect would be depicted as a decreased titer with compound treatment versus virus only.

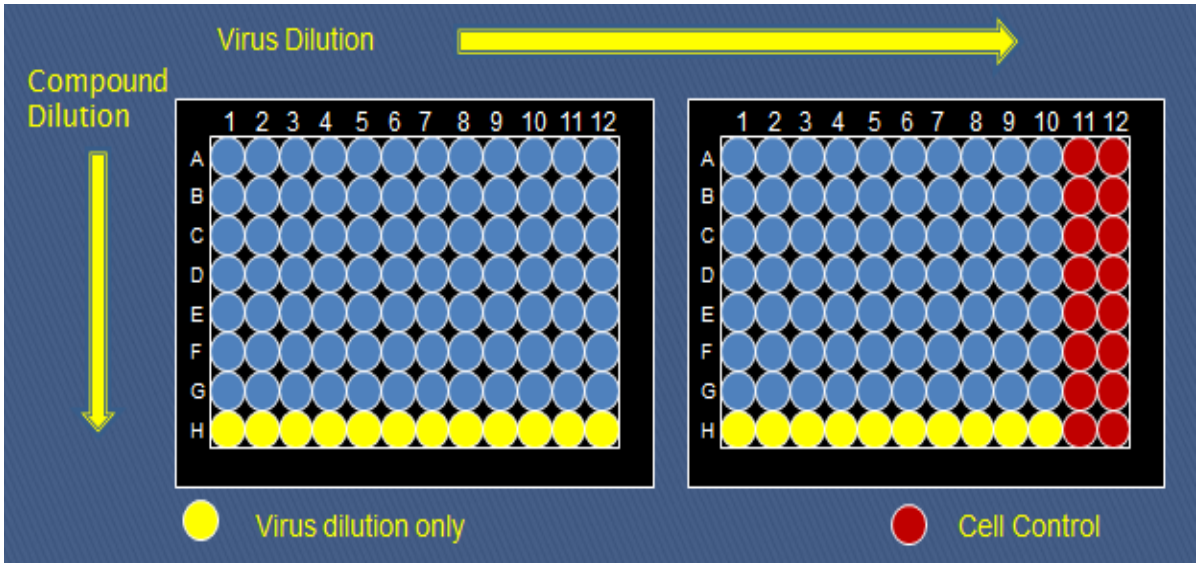
2. Cytotoxicity determination of compounds 5, 7 and 11.

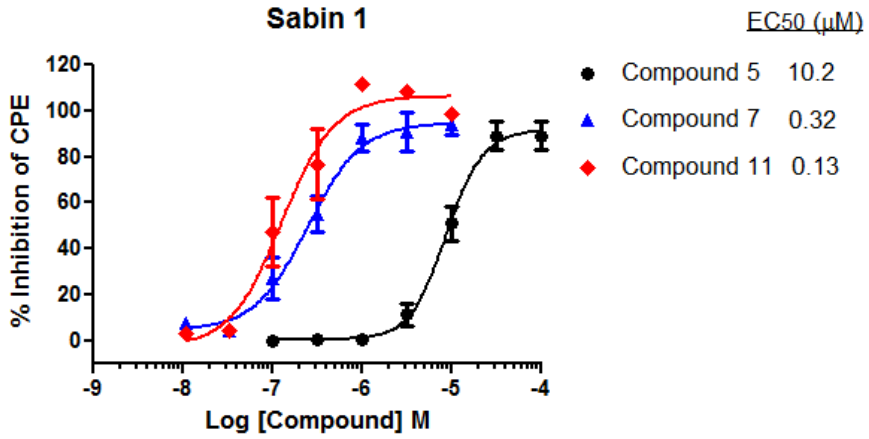


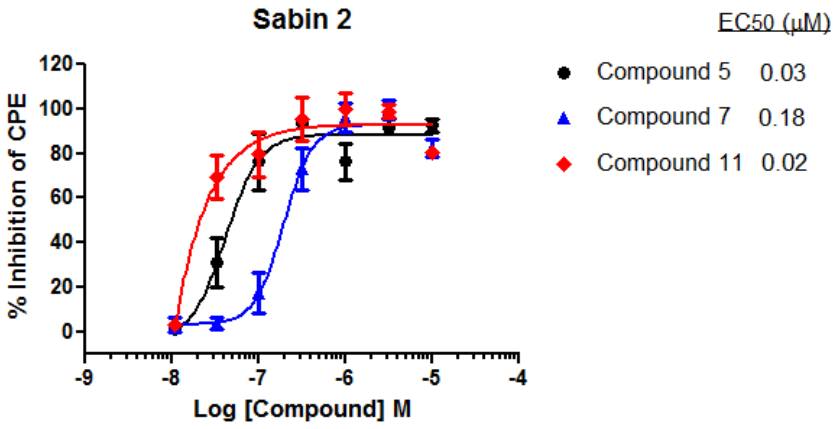


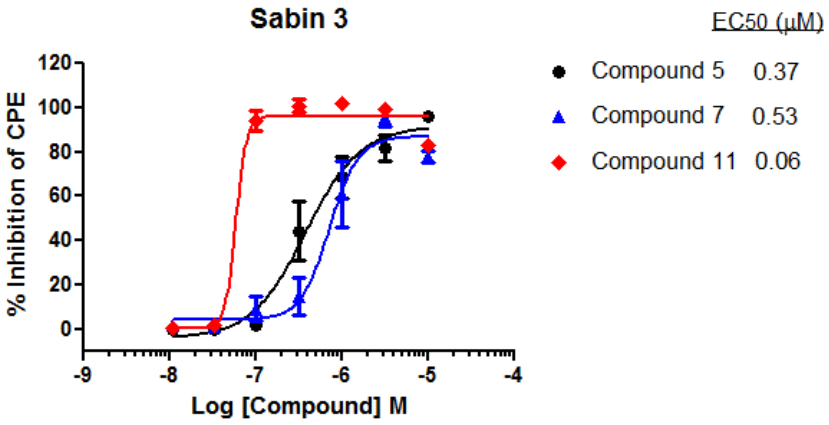
Assay to determine compound cytotoxicity. A luminescence cell viability assay (ATPLite®, Waltham, MA) to determine cytotoxicity was used. Compounds were combined with HeLa cells (2x105 cells/well) in 96-well plates starting at 10 µM compound (100 µM for compounds with EC_{50}>10 µM) (upper panel). Eleven 7-point titration curves were performed in 0.5 log_{10} steps with duplicate wells for each compound concentration. Curves consisting of compound and cells only were compared to cell only wells to determine cytotoxicity. The percentage of cell viability was reported vs Log of compound M (lower panel). After 72 hour incubation and if no cytotoxicity was present, these wells served as the control for calculation of EC_{50}.

3. Determination of EC_{50} for compounds 5, 7, 11 against Sabin 1-3 strains.









Representative titration curves for Sabin 1-3 using an *in vitro* cytopathic effect assay to calculate the susceptibility (EC_{50}, expressed in µM) for a given virus isolate. Compound and virus were combined with HeLa cells ($2x10^5$ cells/well) in 96-well plates, in a cross-titration format, starting at 10 µM compound (100 µM if compound EC_{50} was initially >10 µM) (upper panel). To ensure reaching endpoints from compound and virus titrations, eleven 7-point titration curves were performed in 0.5 log_{10} steps with duplicate wells for each compound-virus concentration. After 72 hour incubation incubation at 37°C, cells were stained with crystal violet (0.05% crystal violet, 0.5% Tween-20, 50% ethanol, in deionized H_2O) and washed three times with deionized H_2O. Plates were air-dried and viral cytopathic effect was measured by reading absorbance at 590 nm. EC_{50} values were derived by analyzing dose-response absorbance values by four-parameter curve fitting using Prism 5.04 (GraphPad Software,Inc., LaJolla, CA) (lower panels). Results represent the mean of 10 EC_{50} curves.